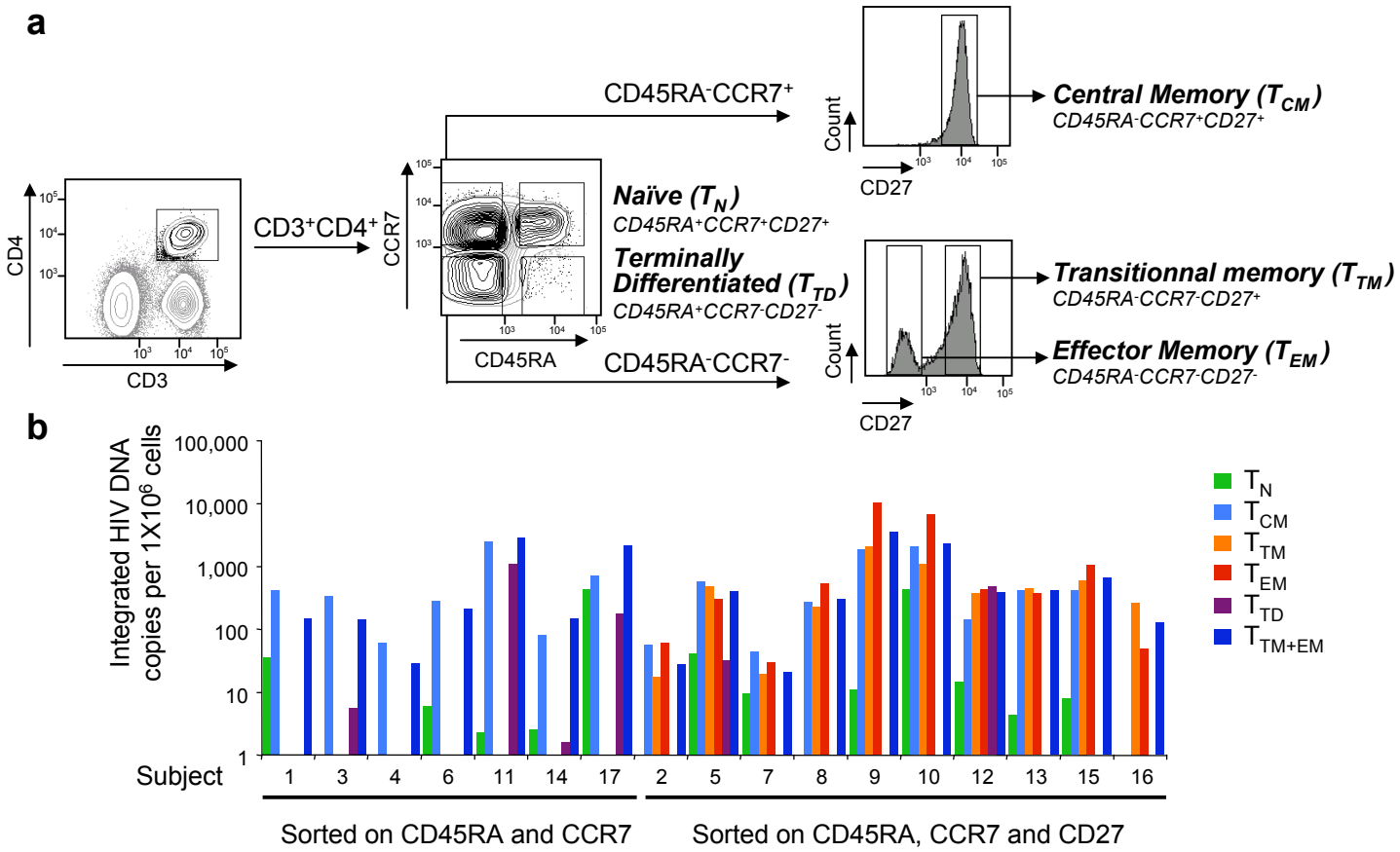
Supplementary information

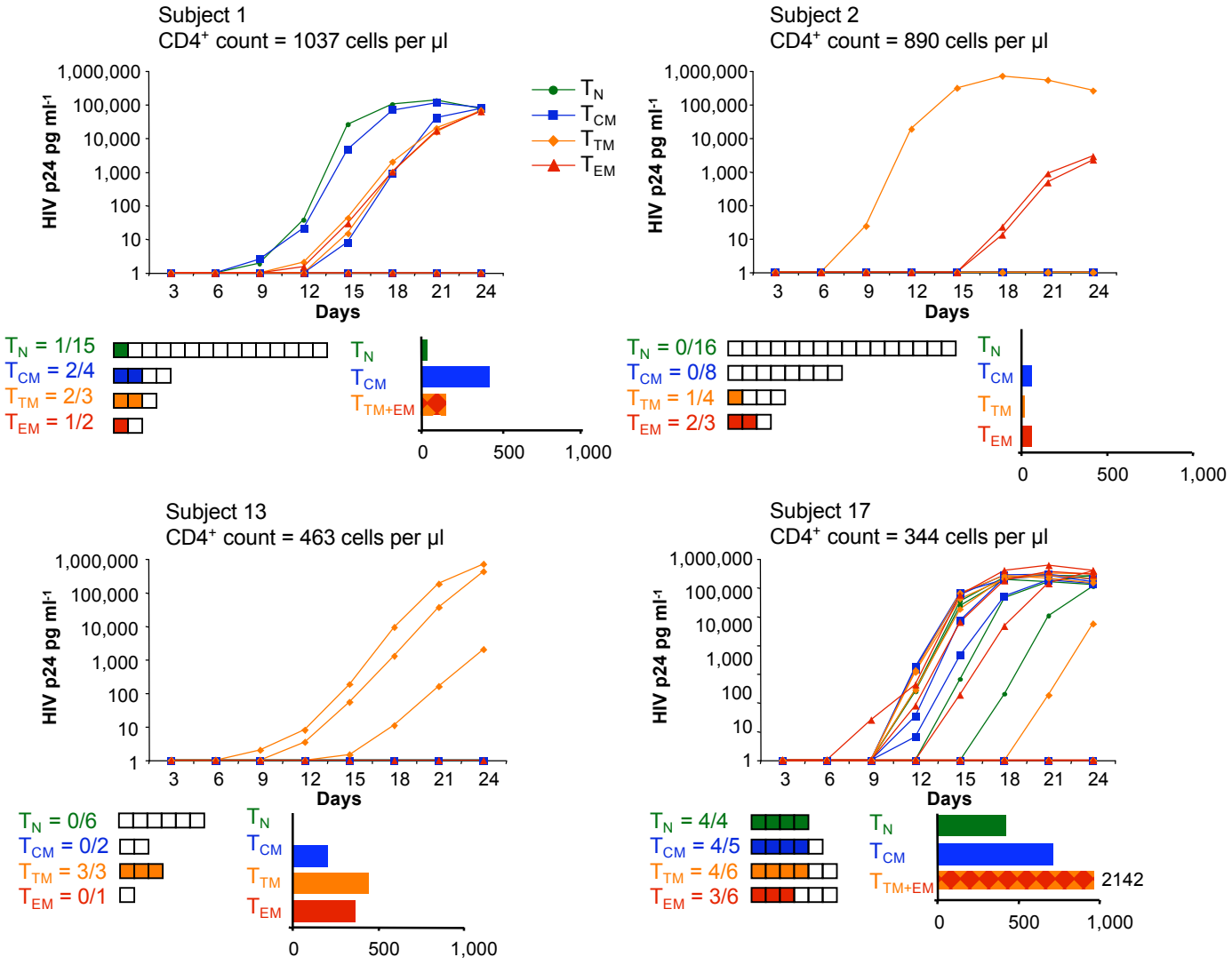
HIV reservoir size and persistence are driven by T-cell survival and homeostatic proliferation.
Chomont, N., M. El Far, P. Ancuta, L. Trautmann, F. A. Procopio, B. Yassine-Diab, G. Boucher, M. R. Boulassel, G. Ghattas, J. M. Brenchley, Timothy W. Schacker, B. J. Hill, D. C. Douek, J. P. Routy, E. K. Haddad, and R. P. Sékaly

Supplementary Figure 1. Gating strategy and quantification of integrated HIV DNA in sorted CD4⁺ T-cell subsets.



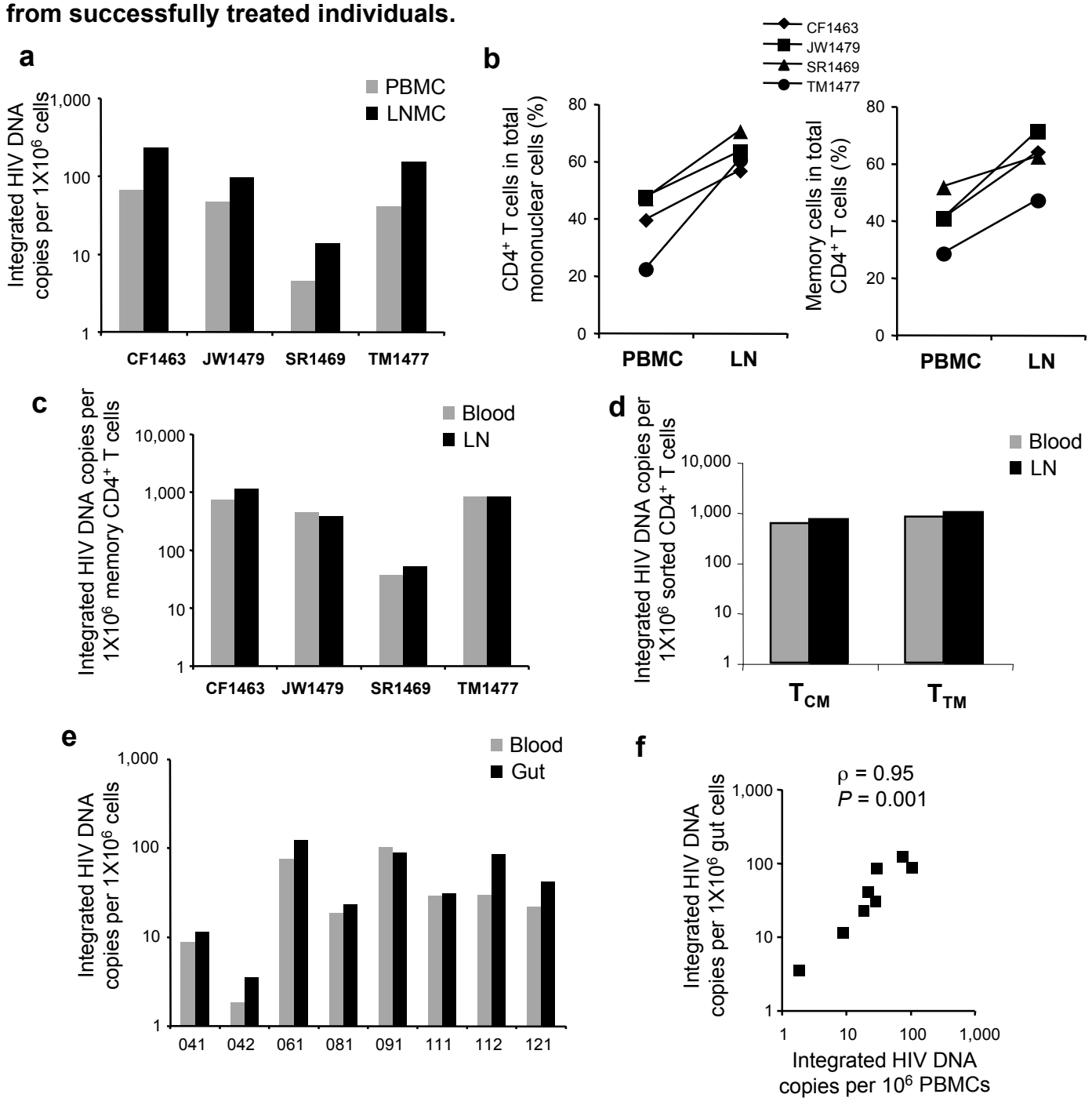
Supplementary Figure 1. Gating strategy and quantification of integrated HIV DNA in sorted CD4⁺ T-cell subsets. (**a**) $CD4^+$ T-cell subsets were isolated after staining with the antibodies indicated in the figure by polychromatic flow cytometry. $CD4^+$ T-cells were sorted according to the expression of CD45RA, CCR7 and CD27. CD27 allows to distinguish between T_{TM} ($CD45RA^-CCR7^-CD27^+$) and T_{EM} ($CD45RA^-CCR7^-CD27^-$), while all T_{CM} express high levels of CD27 ($CD45RA^-CCR7^+CD27^+$). PBMC staining from a representative HIV-infected HAART-treated individual is shown. (**b**) Frequencies of cells harboring integrated HIV DNA in $CD4^+$ T-cell subsets from 17 aviremic subjects. Results are expressed as the HIV copy number in $1X10^6$ cells of a given subset.

Supplementary Figure 2. HIV production following activation of CD4+ T-cell subsets.



Supplementary Figure 2. HIV production following activation of CD4+ T-cell subsets. HIV production was monitored by p24 quantification after PHA/IL-2 stimulation of sorted CD4+ T-cell subsets from 4 individuals with undetectable viral load. Replicates of $5X10^5$ CD4+ T-cells from each subset were stimulated in these conditions. As the number of sorted cells depends on the absolute CD4+ count and on the frequency of each subset in the subjects, we were able to purify 0.5 to $8X10^6$ cells of each subset (1 to 16 independent stimulations). For each subset, the number of positive wells is represented by filled squares. Integrated HIV DNA copy number was determined in all subsets by *alu* PCR in parallel. For subjects 1 and 17, quantification of integrated HIV DNA is given for T_{TM+EM} because the CD27 antibody was not used in all sorting experiments. In subject 1, we recovered infectious virus from all CD4+ T-cell subsets containing integrated HIV DNA. In subject 2, the extremely low frequency of CD4+ T-cells harboring integrated HIV DNA explains the small numbers of p24 positive wells (n = 3/31) observed after stimulation. The limited number of T_{CM} and T_{EM} cells purified from subject 13 (2 and 1 stimulation, respectively) did not allow us to recover infectious virus from these subsets. Conversely, we were able to recover infectious virus from all T-cell subsets in an individual displaying a high frequency of CD4+ T-cells harboring integrated HIV DNA (subject 17).

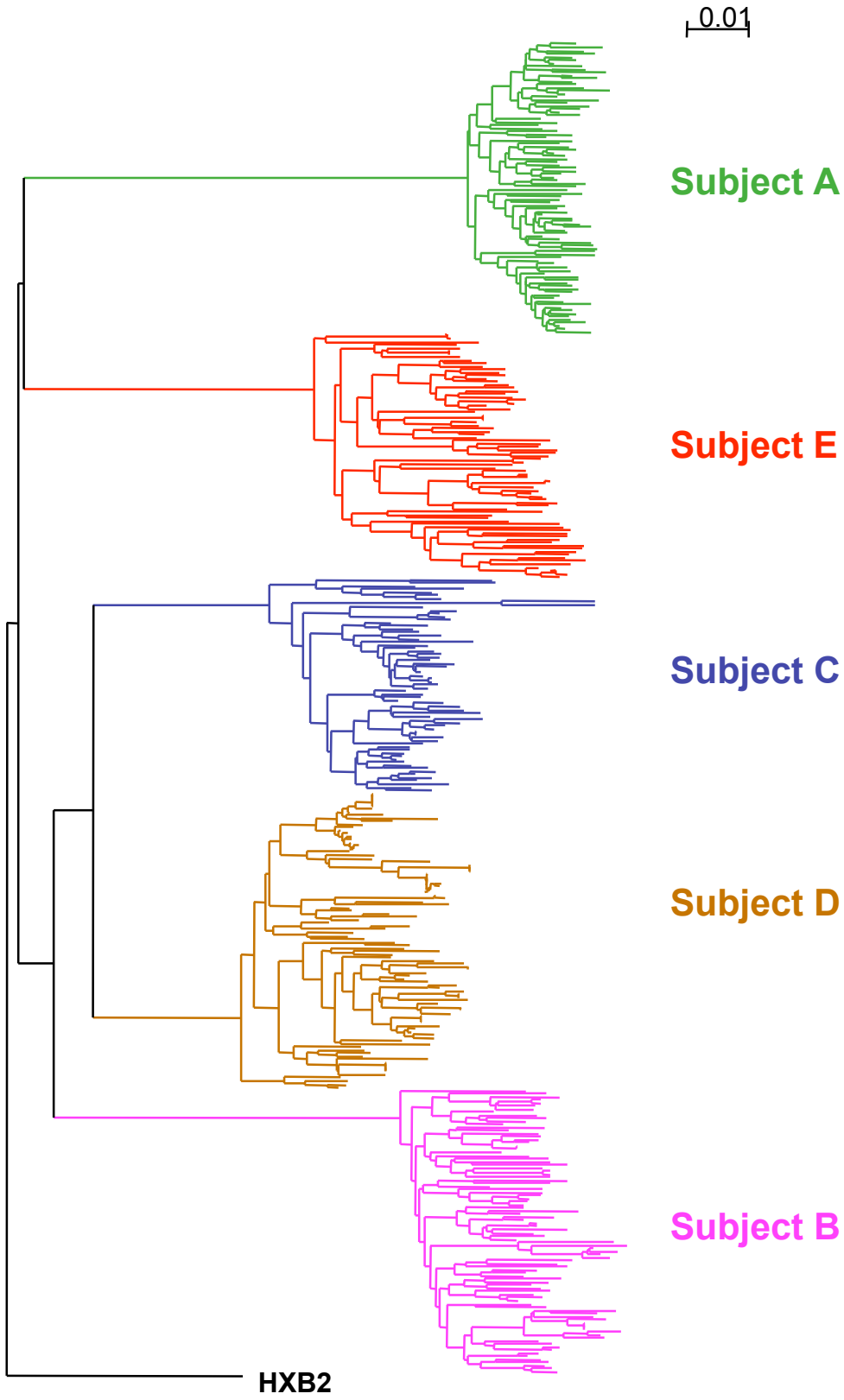
Supplementary Figure 3. Similar infection frequencies in lymphoid tissues and blood from successfully treated individuals.



Supplementary Figure 3. Similar infection frequencies in lymphoid tissues and blood from successfully treated individuals. (**a**) Frequencies of $CD4^+$ T-cells harboring HIV integrated DNA in PBMC and matched LNMC. (**b**) Percentages of $CD4^+$ T-cells in PBMC and LN (left panel) and percentage of memory $CD4^+$ T-cells within the $CD4^+$ compartment (right panel). (**c**) Frequencies of memory $CD4^+$ T-cells harboring HIV integrated DNA in PBMC and matched LN. Results were obtained from infection frequencies in $1X10^6$ PBMC and LNMC after correcting for the relative frequency of memory $CD4^+$ T-cells in each compartment. (**d**) Frequencies of cells harboring integrated HIV DNA in sorted T_{CM} and T_{TM} from LN and blood of a successfully treated subject. (**e**) Frequencies of cells harboring HIV integrated DNA in PBMC and cells from the GI tract in 8 successfully treated individuals. (**f**) Correlation between the frequencies of cells harboring HIV integrated DNA in PBMC and matched gut biopsies.

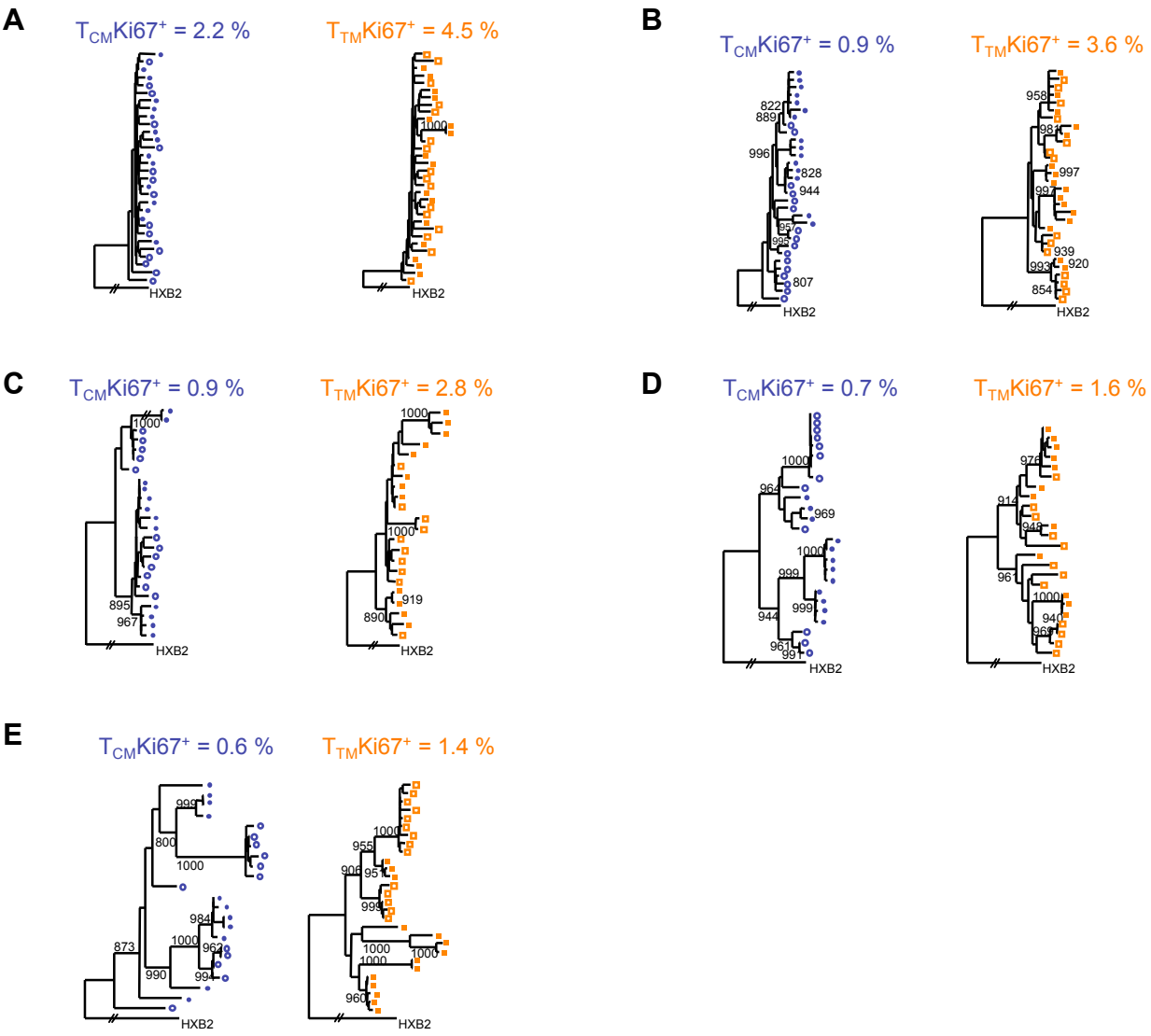
Nature Medicine: doi:10.1038/nm.1972

Supplementary Figure 4. Phylogenetic relationship of viral clones from 5 individuals.



Supplementary Figure 4. Phylogenetic relationship of viral clones from 5 individuals. The unrooted neighbor-joining tree was obtained from the alignment of V1-V3 DNA sequences. The horizontal branches reflect the relative genetic distances between sequences. HXB2 sequence was included as a control.

Supplementary Figure 5. **Evolution of viral sequences in 5 virally suppressed subjects.**



Supplementary Figure 5. Evolution of viral sequences in 5 virally suppressed subjects. Phylogenetic trees derived from HIV sequences obtained from T_{CM} and T_{TM} cells of 5 aviremic subjects (subjects A-E) at first and second time points (closed vs filled symbols, respectively). Percentages of T_{CM} and T_{TM} expressing the Ki67 proliferation marker are indicated.

Supplementary Table 1. Profiles of 34 virally suppressed individuals.

Patient number[1]	Plasma HIV RNA[2] (copies/ml)	CD4+ cell count (cells/mm3)	CD8+ cell count (cells/mm3)	Treatment[3]	Duration of HIV infection (months)[4]	Time of aviremia (months)[5]	HIV reservoir size[6]
1	<50	1037	438	EFV, IDV	54	46	85
2	<50	890	673	3TC, AZT, NEV	57	42	36
3	<50	799	1727	3TC, d4T, NEV	62	33	159
4	<50	691	631	3TC, AZT, IDV	74	67	23
5	<50	671	1120	3TC, ABC, LPV, RIT	242	64	492
6	<50	662	1051	d4T, ddI, EFV	18	13	258
7	<50	602	767	3TC, ABC, SQV	158	53	120
8	<50	599	923	3TC, AZT, EFV	86	46	317
9	<50	563	613	3TC, AZT, IDV	86	71	1408
10	<50	552	715	3TC, d4T, ATZ	139	56	567
11	<50	529	690	3TC, d4T, DEL	49	11	562
12	<50	510	765	3TC, AZT, RIT	61	52	151
13	<50	463	757	3TC, ABC, EFV	152	20	736
14	<50	443	322	3TC, AZT, LPV, RIT	18	12	44
15	<50	424	461	3TC, d4T, DEL	84	46	416
16	<50	356	629	3TC, AZT, ABC	34	6	115
17	<50	344	642	3TC, d4T, NEV	59	44	1135
18	<50	883	333	EFV, IDV	86	78	60
19	<50	834	527	TDF, NEV, ATZ, RIT	38	25	10
20	<50	825	487	3TC, AZT, EFV	16	9	90
21	<50	809	537	3TC, d4T, IDV	71	69	10
22	<50	731	413	3TC, AZT, EFV	51	22	155
23	<50	688	1273	3TC, AZT, EFV	100	59	242
24	<50	663	333	TDF, NEV, ATZ, RIT	55	43	10
25	<50	650	392	3TC, d4T, IDV	64	61	10
26	<50	604	1281	3TC, AZT, IDV	53	35	918
27	<50	501	278	3TC, d4T, IDV	90	87	10
28	<50	499	602	3TC, d4T, ATZ	155	72	352
29	<50	492	582	3TC, ABC, ATZ, RIT	170	66	103
30	<50	463	376	3TC, d4T, NFV	20	9	31
31	<50	434	583	3TC, ABC, EFV	165	34	186
32	<50	396	645	3TC, AZT, ABC	70	42	40
33	<50	337	380	3TC, AZT, EFV	76	68	ND
34	<50	235	399	3TC, d4T, NEV	94	78	817
Mean	**<50**	**594**	**657**		**83**	**45**	**293**

[1] Quantifications of integrated HIV DNA in sorted CD4 T cell subsets were performed in cells from patients 1 to 17.

[2] Viral load were measured by the Amplicor HIV-1 monitor ultrasensitive Method (Roche), with a detection limit of 50 copies/ml of plasma.

[3] Anti-retroviral therapy: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, stavudine; ddI, didanosine; TDF, tenofovir; DEL, delavirdine; EFV, efavirenz; ATZ, atazanavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; NEV, nevirapine; RTV, ritonavir; SQV, saquinavi r

[4] Calculated as the time between the first time point with undetectable viral load and the date of leukapheresis.

[5] Calculated as the time between infection and the date of leukapheresis.

[6] Integrated HIV DNA copy numbers in 10^6 CD4 T cells. ND: not determined.

Nature Medicine: doi:10.1038/nm.1972

Supplementary Table 2. Profiles of 5 subjects followed longitudinally.

	Plasma HIV RNA (copies/ml)[1]	CD4+ cell count (cells/mm^3)	CD8+ cell count (cells/mm^3)	Treatment[1]	CD4+ Ki67+ cells (%)	Duration of HIV infection (months)[1]	Time of aviremia (months)[1]	Time between time points (months)[1]
A	<50	344	642	3TC, d4T, NEV	2.4	59	44	35
	<50	235	399	3TC, d4T, NEV	3.5	94	78	
B	<50	529	690	3TC, d4T, DEL	1.5	49	11	35
	<50	424	461	3TC, d4T, DEL	1.9	84	46	
C	<50	1037	438	EFV, IDV	1.0	54	46	32
	<50	883	333	EFV, IDV	1.5	86	78	
D	<50	599	923	3TC, AZT, EFV	1.6	86	46	14
	<50	688	1273	3TC, AZT, EFV	1.4	100	59	
E	<50	463	757	3TC, ABC, EFV	1.4	152	20	14
	<50	434	583	3TC, ABC, EFV	1 .1	165	34	

[1] Same as in Supplementary Table 1.
[2] Percentage of total CD4 T cell expressing the Ki67 nuclear antigen assessed by flow cytometry.

Nature Medicine: doi:10.1038/nm.1972